CLAIMS

What is claimed is:

1. A method for improving a genetic stability of a foreign insert nucleotide sequence in a recombinant single-stranded RNA virus vector, which comprises performing a mutagenesis of the foreign insert nucleotide sequence (a) to provide even distribution of G/C content throughout the overall foreign insert nucleotide sequence and/or (b) to increase G/C content of the foreign insert without substantially causing amino acid substitutions.

2. The method according to claim 1, wherein the recombinant single-stranded RNA virus vector is derived from one selected from the group consisting of poliovirus, yellow fever virus, Venezuelan equine encephalitis virus, rubella virus and Coxsackie virus.

3. The method according to claim 2, wherein the recombinant single-stranded RNA virus vector is derived from poliovirus.

4. The method according to claim 1, wherein the mutagenesis renders the foreign insert nucleotide sequence to have the G/C content of more than 30%

5. The method according to claim 4, wherein the mutagenesis renders the foreign insert nucleotide sequence to have the G/C content of more than 40%.

6. The method according to claim 1, wherein the mutagenesis of the insert nucleotide sequence to provide even distribution of G/C content is performed by increasing G/C content of local A/T-rich region in the foreign insert nucleotide sequence.

7. The method according to claim 6, wherein the mutagenesis renders the local A/T-rich region of the foreign insert nucleotide sequence to have the G/C content of more than 30%.

8. The method according to claim 7, wherein the mutagenesis renders the local A/T-rich region of the foreign insert nucleotide sequence to have the G/C content of more than 40%.

9. The method according to claim 1, wherein the mutagenesis is performed by silent mutations.

10. The method according to claim 1, wherein the foreign insert nucleotide sequence is smaller than 480 bp in size.

11. The method according to claim 10, wherein the foreign insert nucleotide sequence is smaller than 480 bp in size.

12. The method according to claim 3, wherein the poliovirus is one selected from the group consisting of poliovirus type 1, poliovirus type 2 and poliovirus type 3.

13. The method according to claim 3, wherein the poliovirus is one selected from the group consisting of poliovirus Sabin type 1, poliovirus Sabin type 2 and poliovirus Sabin type 3.

14. The method according to claim 13, wherein the poliovirus is poliovirus Sabin type 1.

15. The method according to claim 1, wherein the foreign insert nucleotide sequence encodes a polypeptide antigen selected from the group consisting of bacterial polypeptide antigens, viral polypeptide antigens, fungal polypeptide antigens and eukaryotic parasite polypeptide antigens.

16. The method according to claim 15, wherein the foreign insert nucleotide sequence encodes a polypeptide antigen of an infectious virus selected from human immunodeficiency

virus, simian immunodeficiency virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, poliovirus, human papilloma virus, herpes simplex virus, rotavirus, influenza virus and epidemic hemorrhagic fever virus.

17. The method according to claim 16, wherein the polypeptide or a protein antigen is derived from the coding region covering the antigenic determinant sites.

18. The method according to claim 15, wherein the foreign insert nucleotide sequence encoding the polypeptide antigen is monomeric, dimeric or multimeric.

19. The method according to claim 18, wherein the dimeric or multimeric foreign insert is homo/hetero-dimmer or homo/hetero-multimer.

20. A method for constructing a recombinant single-stranded RNA virus containing a foreign insert nucleotide sequence, which comprises the steps of:

(a) preparing the foreign insert nucleotide sequence which has an even distribution of G/C content throughout the overall foreign insert nucleotide sequence and/or has a G/C content of more than 30%; and

(b) introducing the foreign insert into a viral genome of a parent RNA virus to construct the recombinant RNA virus,

wherein the foreign insert nucleotide sequence is introduced in such a manner that the the recombinant RNA virus is not disrupted for viral propagation

21. The method according to claim 20, wherein the foreign insert nucleotide sequence comprises a natural-occurring nucleotide sequence.

22. The method according to claim 20, wherein the step of preparing the insert nucleotide is performed by mutagenesis.

23. The method according to claim 20, wherein the recombinant single-stranded RNA virus vector is derived from one selected from the group consisting of poliovirus, yellow fever virus, Venezuelan equine encephalitis virus, rubella

virus and Coxsackie virus

24. The method according to claim 23, wherein the recombinant single-stranded RNA virus vector is derived from poliovirus.

25. The method according to claim 20, wherein the foreign insert nucleotide sequence has the G/C content of more than 40%.

26. The method according to claim 22, wherein the mutagenesis of the foreign insert nucleotide sequence to provide even distribution of G/C content is performed by increasing G/C content of local A/T-rich region of the foreign insert nucleotide sequence.

27. The method according to claim 26, wherein the mutagenesis at a local A/T-rich region renders the region to have the G/C content of more than 30%.

28. The method according to claim 27, wherein the mutagenesis at a local A/T-rich region renders the region to have the G/C content of more than 40%.

29. The method according to claim 22, wherein the mutagenesis is performed by silent mutations.

30. The method according to claim 20, wherein the insert nucleotide sequence is smaller than 480 bp in size.

31. The method according to claim 30, wherein the foreign insert nucleotide sequence is smaller than 450 bp in size.

32. The method according to claim 23, wherein the poliovirus is one selected from the group consisting of poliovirus type 1, poliovirus type 2 and poliovirus type 3.

33. The method according to claim 23, wherein the poliovirus is one selected from the group consisting of poliovirus Sabin type 1, poliovirus Sabin type 2 and poliovirus Sabin type 3.

34. The method according to claim 33, wherein the poliovirus is poliovirus Sabin type 1.

35. The method according to claim 20, wherein the foreign insert nucleotide sequence encodes a polypeptide antigen selected from the group consisting of bacterial polypeptide

antigens, viral polypeptide antigens, fungal polypeptide antigens and eukaryotic parasite polypeptide antigens.

36. The method according to claim 35, wherein the foreign insert nucleotide sequence encodes a polypeptide antigen of an infectious virus selected from the group consisting of human immunodeficiency virus, simian immunodeficiency virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, poliovirus, human papilloma virus, herpes simplex virus, rotavirus, influenza virus and epidemic hemorrhagic fever virus.

37. The method according to claim 36, wherein the polypeptide or a protein antigen is derived from the coding region covering the major or minor antigenic determinant sites.

38. The method according to claim 36, wherein the foreign insert nucleotide sequence encoding the polypeptide antigen is monomeric, dimeric or multimeric.

39. The method according to claim 38, wherein the dimeric or multimeric foreign insert is homo/hetero-dimer or homo/hetero-multimer.

40. A recombinant single-stranded RNA virus comprising an foreign insert nucleotide sequence, characterized in that the recombinant single-stranded RNA virus is constructed by the method according to claim 20.

41. The recombinant single-stranded RNA virus according to claim 40, wherein the foreign insert nucleotide sequence is smaller than 480 bp in size.

42. The recombinant single-stranded RNA virus according to claim 41, wherein the foreign insert nucleotide sequence is smaller than 450 bp in size.

43. The recombinant single-stranded RNA virus according to claim 40, wherein the poliovirus is one selected from the group consisting of poliovirus type 1, poliovirus type 2 and poliovirus type 3.

44. The recombinant single-stranded RNA virus according to claim 43, wherein the poliovirus is one selected from the group consisting of poliovirus Sabin type 1, poliovirus Sabin type 2 and poliovirus Sabin type 3.

45. The recombinant single-stranded RNA virus according to claim 44, wherein the poliovirus is poliovirus Sabin type 1.

46. The recombinant single-stranded RNA virus according to claim 40, wherein the foreign insert nucleotide sequence encodes a polypeptide antigen selected from the group consisting of bacterial polypeptide antigens, viral polypeptide antigens, fungal polypeptide antigens and eukaryotic parasite polypeptide antigens.

47. The recombinant single-stranded RNA virus according to claim 46, wherein the foreign insert nucleotide sequence encodes a polypeptide antigen of an infectious virus selected from human immunodeficiency virus, simian immunodeficiency virus, hepatitis A virus, hepatitis B virus, hepatitis C virus, poliovirus, human papilloma virus, herpes simplex virus, rotavirus, influenza virus and epidemic hemorrhagic fever virus.

48. The recombinant single-stranded RNA virus according to claim 47, wherein the polypeptide or the protein antigen is derived from the coding region covering the major or minor antigenic determinant sites.

49. The recombinant single-stranded RNA virus according to claim 47, wherein the foreign insert nucleotide sequence encoding the polypeptide antigen is monomeric, dimeric or multimeric.

50. The recombinant single-stranded RNA virus according to claim 49, wherein the dimeric or multimeric foreign insert is homo/hetero-dimer or homo/hetero-multimer.

51. The recombinant single-stranded RNA virus according to claim 43, wherein the recombinant poliovirus comprises:

(a) a genomic nucleotide sequence of a parent poliovirus;

(b) an additional polioviral cleavage site; and

(c) the foreign insert nucleotide sequence,

wherein the foreign insert nucleotide sequence is introduced into the viral genome of a parent poliovirus without disrupting the viral infection and proliferation, and a poliovirus protease also acts on the additional cleavage site so that the polypeptide or protein antigen encoded by the foreign insert nucleotide sequence is released from a polyprotein precursor of the recombinant poliovirus.

52. A method for amplifying a nucleotide sequence using template/ligation-free PCR method, which comprises the steps

of:

(a) preparing a plurality of DNA fragments serving as both template and primer, in which the DNA fragments are designed by dividing the entire nucleotide sequence of interest into several fragments with different complementary regions so that one segment is used as a template while being primed by the other;

(b) mixing the DNA fragments in such as manner that the DNA fragments corresponding to both ends of final nucleotide sequence which is also used in amplification step has a higher concentration than the other DNA fragments;

(c) preparing a full length of the nucleotide sequence of interest by PCR for 20-40 sec at 92-96℃ (denaturation), for 20-40 sec at 25-40℃ (annealing) and for 40-70 sec at 68-75℃ (extension); and

(d) amplifying the nucleotide sequence of interest by PCR for 20-40 sec at 92-96℃ (denaturation) and 40-70 sec at 68-75℃ (annealing and extension).

53. The method for amplifying a nucleotide sequence according to claim 52, wherein the concentration ratio of the DNA fragments corresponding to both ends of final nucleotide sequence to the other DNA fragments is 1:3-1:8.

54. The method for amplifying a nucleotide sequence according to claim 52, wherein the complementary regions in the DNA fragments have 8-20 mer of length and G/C content of more than 35%.

55. The method for amplifying a nucleotide sequence according to claim 52, wherein the DNA fragments corresponding to both ends of final nucleotide sequence have a cloning site comprising restriction sites.